IKZF1 AND RISK OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN WITH DOWN SYNDROME

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Background: Down syndrome (DS) results from trisomy 21 and is one of the strongest genetic risk factors for acute lymphoblastic leukemia (ALL). Children with DS have a 20 times greater risk of developing ALL than other children. We performed the first genome-wide association study (GWAS) of ALL in children with DS and identified a risk locus at IKZF1, a master regulator of B cell development. IKZF1 risk alleles identified in previous GWAS of non-DS ALL and associated with decreased IKZF1 expression. Here, we examined the functional effects of IKZF1 variants on B cell development using genome editing in patient-derived lymphoblastoid cell lines (LCL) and bone marrow hematopoietic stem cells (HSC) from our Dp16 mouse model of DS.

Materials/Methods: Allele-specific reporter and protein binding assays were performed in LCLs with the IKZF1 non-risk haplotype. Genome editing was performed by transducing cells with lentivirus expressing all-in-one CRISPR vectors. Genome editing in monoclonal populations was confirmed by PCR and amplicon sequencing. Expression of IKZF1 was assayed by qPCR.

Results: Functional annotation of our top SNP rs58923657 shows this genomic locus is a B cell super-enhancer, suggesting this region influences IKZF1 expression in a cis-regulatory manner. We further prioritized candidate SNPs using ENCODE data and functional reporter assays. We observed a significant decrease in enhancer activity ($P=0.004$), and differential protein binding ($P<0.05$) associated with the risk allele. Furthermore, we show rs17133807 exhibits a unique combination of evolutionary conservation, enhancer localization, and differential protein binding, thus indicating the best causal SNP candidate. To replicate the effects of the risk allele, we used CRISPR to knockout (KO) the orthologous rs17133807 enhancer in Dp16 HSCs. We found a significant decrease in Ikzf1 expression in KO-HSCs compared to untreated cells ($P<0.01$), supporting a causal role for rs17133807.

Conclusions: Here, we report an experimental follow-up to identify the molecular mechanism driving the IKZF1 association with increased risk for DS-ALL. We provide functional evidence utilizing a combination of in vitro reporter assays and CRISPR/Cas9 technology. Our data is compatible with the IKZF1 risk locus conferring increased risk for DS-ALL by disrupting IKZF1 enhancer activity. Next, we will utilize CRISPR/Cas9 genome editing in patient-derived LCLs and cord blood HSCs to investigate the effects of the IKZF1 risk allele on human B cell development.